3268-Plat
A Conserved Water-Mediated Hydrogen Bond Network Underlies Selectivity of the Kinase Inhibitor Bosutinib
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Kinase inhibitors are anti-cancer drugs developed to target particular oncogenic protein kinases, but these molecules display many off-target effects. The development of more selective compounds is hindered by our poor understanding of how sequence variation among kinases affects interactions with inhibitors. Here we show that the clinical kinase inhibitor bosutinib exploits a conserved water-mediated hydrogen bond network when it binds to target kinases, and that this recognition mechanism is a widespread feature of type I kinase inhibitors. We used the nitrile group of bosutinib as a site-specific infrared probe to study how substitutions in the ATP-binding site modulate the nitrile's engagement in the hydrogen bond network. The particular amino acids found at the gatekeeper and one other position exert exclusive control, contributing ~2 kcal/mol to selective binding, and the selectivity profile of the drug across the human kinome can be systematically rationalized in terms of the impact of these substitutions on the hydrogen bond network. Our work highlights the key importance of structured water molecules for inhibitor recognition, reveals a new mechanism by which the gatekeeper can control inhibitor selectivity, and showcases an effective approach for elucidating the molecular origins of selectivity.

3269-Plat
Molecular Mechanisms Underlying the Clinical Success of the Cancer Drug Gleevec
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Cancer is a serious illness and the second leading cause of death in the US. It is caused by misregulation of the signaling cascades and uncontrolled cell proliferation. Protein kinases are the key players in the regulatory machinery, and in the last 20 years they have become an increasingly important drug targets. The main requirement for such drugs is selectivity: ideally, only one kinase should be affected by an individual drug. The prominent success story is an FDA-approved chronic myeloid leukemia therapeutic Gleevec. Gleevec is a highly specific inhibitor of the Abl kinase and has very little potency towards a closely related Src subfamily of kinases. This specificity is particularly intriguing considering that the drug-binding pockets look nearly identical in these kinases. We combined NMR and pre-steady-state kinetic experiments to monitor Gleevec binding in real time and with residue-specific precision. Our data establish a novel model that fully and quantitatively explains the observed 3000-fold difference in Gleevec's affinities to Abl and Src, solving a long-standing paradox in the field. These results reveal the general principles of kinase-drug interactions and highlight the molecular mechanisms behind nanomolar affinities found in clinically relevant therapeutics.

3270-Plat
Finding Hidden Allosteric Sites in Proteins
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The substantial dynamics that folded proteins undergo creates the potential for hidden allosteric sites-unexpected pockets capable of altering a protein's activity via long-range communication with the active site-that could be valuable drug targets. Unfortunately, identifying these sites and targeting them with drugs remains a profound challenge, typically requiring resource intensive screening of large libraries of small molecules. Here, I will present a combination of computational and experimental tools for identifying hidden allosteric sites without having to consider specific ligands. First, we use atomically-detailed Markov state models of a ligand-free protein to identify potential allosteric sites based on two signature structural fluctuations: *(i)* the presence of a pocket in a non-trivial fraction of the protein population and *(ii)* the presence of correlated motions allowing communication between residues surrounding the pocket and the active site where chemistry is performed. Second, we use labeling experiments to test the existence of proposed pockets. Application of this approach to TEM-1 β-lactamase-an important drug target for overcoming antibiotic resistance-demonstrates that these methods are capable of recovering known hidden allosteric sites. Moreover, our approach reveals a new allosteric site in β-lactamase that may serve as a valuable drug target. Therefore, we propose our methods will be a powerful means of identifying additional hidden allosteric sites in other proteins.

3271-Plat
Computational Prediction of Protein-Peptide Binding
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Protein-peptide interactions are crucial for many important biological processes, especially during signal transduction, as they regularly trigger signaling by initiating molecular recognition events. Additionally, peptides are natural inhibitors for proteins and therefore important lead structures in pharmaceutical research. Prominent examples for peptide-based drugs are inhibitors of viral proteases [1, 2].
There exist very few computational approaches, which allow a structure-based prediction of protein-peptide binding, especially for larger peptides (> 5 amino acids) and surface-exposed binding sites. We have developed a two-stage method for this purpose.
During our procedure, we first predict the peptide's binding site on the protein's surface. This step is important, as for many biologically relevant protein-peptide interactions no structural information is available for the bound complex. Afterwards we sample all possible peptide conformations in the predicted binding site to identify the bound conformation of the protein-peptide complex using two methods: IRECS [3, 4], an algorithm for predicting side chain conformations, and DynaDock[5], a molecular dynamics-based docking approach, which allows an efficient description of the protein's flexibility during protein-peptide assembly.
The methodology was successfully evaluated in various projects e.g. investigating peptide binding to Hsp70 and MHC proteins [6]. We will give an outline of the methodology and its application.
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3272-Plat
Rastering the Influenza Virus Surface with Molecular Rulers and Nanoparticles to Design Optimal Multivalent Inhibitors
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Multivalency is a widespread phenomenon in many biological systems, and inevitable for specific surface-surface interactions. The influenza virus provides a suitable system for studying multivalent interactions as it is covered by a densely packed layer of hemagglutinin (HA), which provides the virus high affinity to the host cells glycocalyx by multivalent interactions. To prevent such interactions and thus, infection, one promising strategy is the use of inhibitors presenting multiple HA receptors. For efficient inhibition, we investigated the optimal spatial arrangement, and amount of ligands of multivalent structures, among others multivalent polyglycerols and DNA-PNA heteroduplexes. Natural receptors such as sialic acid or peptide fragments from HA antibodies, form the basis of our multivalent constructs.
In order to determine exact distances between HA binding sites, we introduced bivalent DNA-PNA heteroduplexes, presenting sialic acid with distinct distances, and thus serving as molecular rulers. Inhibition of fluorescent virions to red blood cells by heteroduplexes was assessed by flow cytometry measurement. By this strategy, we determined the spatial arrangement of sialic acid for optimal binding to virus at angstrom resolution. Microscale thermophoresis (MST) technology served as an independent and complementary approach to assess binding. Here, we detected nanomolar dissociation constants to full virus particles free in solution. In some instances, we observed multiphasic binding kinetics of multivalent structures to influenza virions. These studies are not only highly relevant for inhibition of virus infection, but also to provide new insights on the fundamentals of multivalent interactions.